

Placental pathology, birthweight discordance, and growth restriction in twin pregnancy: results of the ESPRiT Study

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OBJECTIVE: We sought to evaluate the association between placental histological abnormalities and birthweight discordance and growth restriction in twin pregnancies.

STUDY DESIGN: We performed a multicenter, prospective study of twin pregnancies. Placentas were examined for evidence of infarction, retroplacental hemorrhage, chorangioma, subchorial fibrin, or abnormal villus maturation. Association of placental lesions with chorionicity, birthweight discordance, and growth restriction were assessed.

RESULTS: In all, 668 twin pairs were studied, 21.1% monochorionic and 78.9% dichorionic. Histological abnormalities were more frequent in placentas of smaller twins of birthweight discordant pairs ($P = .02$)

and in placentas of small for gestational age infants ($P = .0001$) when compared to controls. The association of placental abnormalities with both birthweight discordance and small for gestational age was significant for dichorionic twins ($P = .01$ and $.0001$, respectively). No such association was seen in monochorionic twins.

CONCLUSION: In a large, prospective, multicenter study, we observed a strong relationship between abnormalities of placental histology and birthweight discordance and growth restriction in dichorionic, but not monochorionic, twin pregnancies.

Key words: birthweight discordance, placental infarcts, placental pathology, retroplacental hematoma, subchorial fibrin, twin pregnancy

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Rates of twin pregnancies are rising as assisted reproductive techniques have become ever more advanced.¹ Twin pregnancies have significantly higher

rates of perinatal morbidity and mortality than singleton pregnancies.² While this is, to a large extent, related to the high rate of preterm delivery in these pregnancies, the excess of fetal growth abnormalities is also significant.³ Independently of gestational age at delivery, twins with significant birthweight discordance have poorer perinatal outcomes.^{4,5}

The etiology of birthweight discordance in twins has been extensively investigated. In monochorionic twins, differences are largely attributed to twin-to-twin transfusion syndrome, inequalities in distribution of placental mass between the 2 fetuses, and abnormalities in cord insertion site.^{6,7} In dichorionic twins there may be a difference in genetic growth potential in certain cases, but frequently growth discordance is a pathological entity leading to adverse neonatal outcomes.⁵

Placental pathological examination at both a gross and microscopic level is useful in informing our knowledge of the etiology of abnormal growth in both singleton and twin pregnancies, with a variety of placental pathological lesions implicated in intrauterine growth restriction.⁸

In a large prospective cohort of twin pregnancies we evaluated the association

of placental pathology with twin growth restriction.

MATERIALS AND METHODS

The Evaluation of Sonographic Predictors of Restricted Growth in Twins (ESPRiT) Study was a prospective, multicenter, observational study of twin pregnancies carried out by the Perinatal Ireland Research Consortium at 8 tertiary-level obstetric units in Ireland from May 2007 through October 2009. The ESPRiT Study was set up with the primary aim of establishing a level of birthweight discordance in twin pregnancies that would serve as an independent predictor of adverse perinatal outcome. The study had a number of prespecified secondary analyses including the evaluation of the role of placental pathology in the etiology of birthweight discordance and restricted growth in twin pregnancies. Institutional review board approval was obtained in each center and participants gave written informed consent, including consent to placental histological examination after delivery.

Inclusion criteria for the study were twin pregnancies enrolled <22 weeks' gestation, with both twins alive at the time of enrollment and intact membranes. Monoamniotic twins were ex-

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TABLE 1
Clinical characteristics

Variable	Monochorionic	Dichorionic	P value
Total, n (%)	141 (21.1)	527 (78.9)	
Mean BW, g	2201 (632)	2504 (567)	< .0001
Mean GA at delivery, wk	34.7 (2.9)	36.3 (2.4)	< .0001
BW discordance >20%	26 (18.4)	88 (16.7)	0.6
BW <5th centile	16 (5.7)	76 (7.2)	0.4
Composite perinatal morbidity	83 (29.4)	150 (14.23)	< .0001

Data are expressed as n (% of total) or mean (SD).
BW, birthweight; GA, gestational age.

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cluded, as were cases where one or both twins had a major structural or a chromosomal abnormality.

Following enrollment all subjects underwent intensive sonographic surveillance with regular assessment of biometric parameters as well as multivessel Doppler studies. Delivery outcome data were collected including mode of delivery, gestational age, and birthweight. Perinatal morbidity outcomes that were assessed were death, respiratory distress syndrome, hypoxic-ischemic encephalopathy, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis, and sepsis.

Following delivery placentas were collected and labeled as A (1 cord clamp) or B (2 cord clamps) according to birth order. Placental examination was carried out in the pathology department of the delivery hospital according to a defined study protocol, as outlined below. Pathologists were not blinded to clinical outcomes. Where there was no patholo-

gist available to carry out the placental examination locally in accordance with the study protocol, the placental examination was carried out in the pathology department of the Rotunda Hospital, Dublin, the coordinating hospital for the study.

Formalin fixation was carried out as per local practices in the delivery hospital. Placentas undergoing fixation were immersed in 10% phosphate-buffered formalin for a minimum of 24 hours.

Gross placental examination was performed to evaluate umbilical cord vessel number and umbilical cord insertion site. Evaluation of placental chorionicity was performed by examination of the intertwin membrane. Both gross and histological examinations of the intertwin membrane were performed and each twin pregnancy was recorded as dichorionic or monochorionic.

The membranes were sampled at the rupture site and at a further random site and examined histologically. A cross sec-

tion of each umbilical cord from the placental end and the fetal end was submitted for microscopy. Each placental disc was examined macroscopically and multiple sections from each disc were submitted for histological assessment. When examining the monochorionic placentas the vascular equator was identified and the placental mass on either side of this was considered separately and the findings were assigned to the corresponding twin. The following placental pathological lesions were recorded: placental infarction, chorangioma, subchorial fibrin deposition, and retroplacental hematoma. All lesions identified on gross examination were sampled for histological assessment. The size of the lesion was recorded and its location relative to twin 1 or twin 2. The presence of histological abnormalities in placental villus maturation was noted for either twin in each twin pair. The composite outcome of placental abnormalities used in the final analysis was the presence of ≥ 1 of the placental lesions described.

Birthweight discordance was calculated for each twin pair by expressing the absolute difference in birthweight as a percentage of the birthweight of the larger twin. For the purposes of this analysis a discordance level of $\geq 20\%$ was deemed significant. Twins were classified as appropriate for gestational age (AGA) or small for gestational age (SGA) by plotting birthweights on twin-specific birthweight centiles.⁹ SGA was defined as birthweight <5th centile for gestational age.

The frequency of placental pathological lesions was compared for monochorionic and dichorionic twin pregnancies. The composite placental pathology was then assessed as a factor in birthweight discordance and growth restriction. Frequency of occurrence was compared between smaller twins of birthweight discordant pairs and the larger co-twins and concordant controls. The relative frequency was also analyzed between twins with birthweight <5th centile for gestation and those appropriately grown. Analyses were stratified by chorionicity.

Statistical analyses were performed using SAS software (version 9.1; SAS Institute, Cary, NC). Relative frequencies were compared using χ^2 test. Paired stu-

TABLE 2
Frequency of placental pathological lesions

Variable	Monochorionic	Dichorionic	P value
Infarction	34 (18.7)	88 (8.3)	.0001
Chorangioma	0 (0)	7 (0.7)	.6
Subchorial fibrin	32 (17.6)	113 (10.7)	.012
Retroplacental hematoma	17 (9.3)	53 (5.0)	.03
Abnormal villus maturation	53 (29.1)	151 (14.3)	.0001
Composite placental outcome	117 (41.5)	347 (32.9)	.009

Data are expressed as n (% of total).

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dent *t* test was used to analyze continuous variables. A *P* value of $< .05$ was considered statistically significant.

RESULTS

Of 1001 twin pairs recruited to the ESPRiT Study, 66.7% ($n = 668$) had complete placental pathological examination data available for analyses. Of these, 21.1% ($n = 141$) were monochorionic and 78.9% ($n = 527$) dichorionic. Table 1 illustrates the clinical characteristics of the cohort. Monochorionic twins were delivered at an earlier mean gestational age and were on average 302 g lighter than their dichorionic counterparts. The relative frequency of both birthweight discordance of $\geq 20\%$ and birthweight < 5 th centile was not statistically significantly different between the 2 groups. A composite measure of adverse perinatal outcome, which included any of the morbidity measures described above or perinatal death, was more frequent in monochorionic twins.

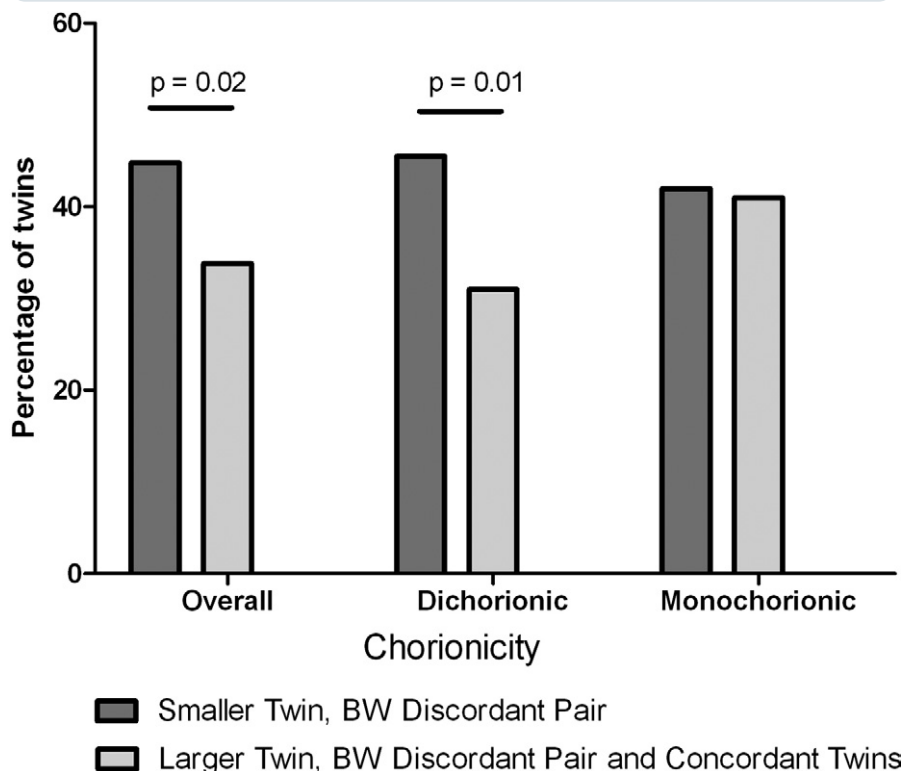
Overall 34.7% ($n = 464/1336$) of twins in the study group had a placenta that demonstrated ≥ 1 of the placental pathological lesions assessed. Lesions were more frequently seen in placentas of monochorionic than dichorionic twins ($P = .009$) (Table 2). When the individual histological abnormalities were categorized all abnormalities other than chorangioma were significantly more common in monochorionic placentas (Table 2).

The relationship between birthweight discordance and placental abnormalities was then analyzed. Overall 17.1% ($n = 114$) of the cohort had $> 20\%$ difference in birthweight between the smaller and larger twin. The results for the smaller discordant twins ($n = 114$) were compared to the combined group of larger co-twins and twins with concordant birthweight ($n = 1222$). In all, 44.7% of the smaller twins had abnormal findings at placental examination. This was significantly more frequent than the comparison group of larger twins and twins with concordant birthweight (33.8%, $P = .02$) (Figure 1).

These results were then stratified by chorionicity to ascertain if this relation-

FIGURE 1

Relative frequency of placental histological abnormalities in BW discordant twin pairs



BW, birthweight.

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ship was present in both monochorionic and dichorionic twins (Figure 1). A significant association between abnormalities in the placenta and birthweight discordance was found in the dichorionic cohort, with a higher frequency of placental pathological lesions in the smaller twins, but a similar association was not ascertained among monochorionic twins.

A further analysis of the results was performed to determine the correlation between fetal growth restriction and placental pathology, comparing twins with birthweight < 5 th centile for gestational age (SGA) and those with birthweight > 5 th centile (AGA). Of the 6.7% ($n = 90$) of SGA babies within the cohort, 57.8% ($n = 52$) had evidence of ≥ 1 of the described abnormalities of placenta-tion. This was almost twice as frequent as for AGA ($n = 412/1246$, 33.1%, $P = .0001$).

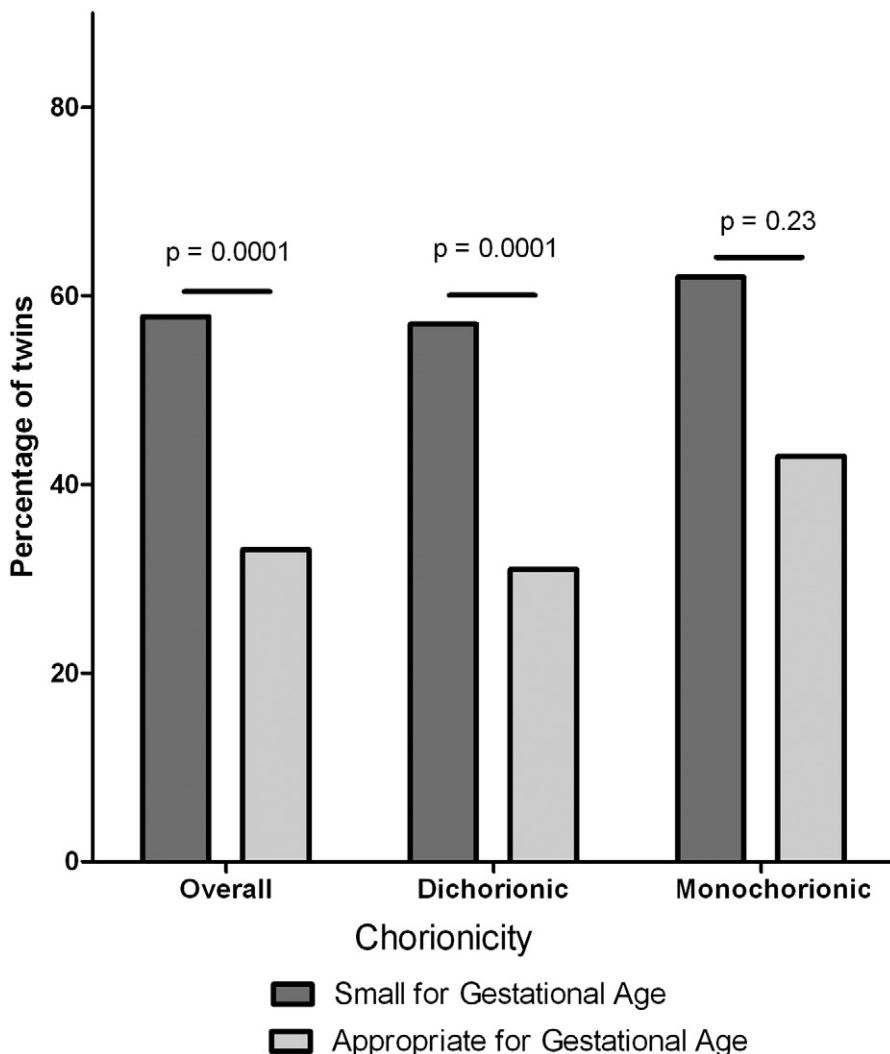
When further analyzed separately according to chorionicity the association

between SGA status and placental pathology was significant for dichorionic, but not monochorionic, twins ($P = .0001$ and $.23$, respectively) (Figure 2), although the latter analysis was limited by the relatively low number of SGA monochorionic twins ($n = 16$) in our study cohort.

COMMENT

It is now well established that twin pregnancies with evidence of growth discordance are at increased risk for perinatal mortality and morbidity.⁵ Placental histological examination is vital in the evaluation of intrauterine growth restriction. Studies in singletons have shown morphological changes in villus structure in placentas of fetuses affected by intrauterine growth restriction.^{8,10,11} The abnormalities in villus development are a consequence of defective trophoblast invasion and impaired development of the uteroplacental circulation.

FIGURE 2
Relative frequency of placental histological abnormalities
in small and appropriate for gestational age twins



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Uteroplacental vascular disease has also been associated with placental infarcts in singleton pregnancies complicated by intrauterine growth restriction.¹²

Previous studies in twin pregnancies have evaluated placental disease as a contributor to birthweight discordance.¹³⁻¹⁵ Most of the studies to date have concentrated on the monochorionic placenta evaluating the abnormal vascular relations at play in the pathogenesis of twin-to-twin transfusion syndrome.¹⁶⁻¹⁸ Several authors have also evaluated umbilical cord abnormalities and placental weight in the occurrence of discordant growth in twins.¹⁹⁻²¹ However, there is a paucity of

data in the literature on the gross and histological placental findings in monochorionic and dichorionic twins.

Studies published previously evaluating similar outcomes have been limited by relatively small numbers.^{14,15} Inconsistencies in findings in these previous studies may be related to their size and their retrospective nature. In our study the protocol for placental evaluation was established prior to commencing the study, and distinct pathological lesions were identified as potential etiological factors in abnormal growth of twin pregnancies, thus minimizing variations in reporting of placental pathology outcomes.

In a prospectively assessed cohort of 668 twin pregnancies we have established associations between placental pathological lesions and suboptimal growth. In dichorionic twins we found that both discordant birthweight and SGA status were significantly associated with underlying abnormalities in placental histological findings. Monochorionic twin pregnancies did not show a similar association.

As expected, we found that overall babies with a birthweight <5th centile for gestation had significantly higher rates of placental abnormalities than appropriately grown controls. When stratified by chorionicity this finding held true only within the dichorionic cohort. However, there were only 16 SGA babies in the monochorionic cohort and therefore it is likely that the study was underpowered to detect a significant difference in this group.

Two previous publications in the literature assessed similar outcomes. A retrospective analysis of 147 twin placentas by Eberle et al¹⁴ evaluated various placental pathological lesions and found that discordant birthweight in dichorionic twins was related to increased numbers of placental lesions in lighter twins of discordant pairs. In a similar finding to our study this association was not present in their monochorionic twin cohort. This study was limited, however, by very small numbers, with only 48 monochorionic twin pairs included.

A study by Victoria et al¹⁵ included 388 dichorionic and 89 monochorionic twin pregnancies. A retrospective analysis of the placental pathology charts in this cohort showed significantly more vascular thrombotic lesions in the placental domains of smaller twins in discordant monochorionic pairs. However, a similar association was not seen in the placentas of discordant dichorionic twins.

We have previously published outcomes from the ESPriT cohort relating to cord insertion site and its relation to both birthweight discordance and SGA status.¹⁹ The contrast in findings of our 2 studies adds significantly to our knowledge of the differing pathological processes at play in monochorionic and di-

chorionic twin pregnancies complicated by growth abnormalities. In our prior study we established a significant association between noncentral placental cord insertion sites and growth abnormalities in monochorionic twins. A similar association was not seen for dichorionic twins. In direct contrast the results presented here have established that for dichorionic twins growth discordance may be related to underlying uteroplacental insufficiency selectively affecting 1 twin. While there was a high frequency of placental abnormalities observed in monochorionic twins this did not appear to influence the occurrence of birthweight discordance. This finding suggests that any placental lesions in monochorionic twin pregnancies are evenly distributed across the placental disc. The occurrence of significant growth discordance in these pregnancies is likely a factor of an unequal distribution of placental mass between the 2 fetuses, abnormal cord insertion sites, or aberrant vascular connections.

Our study has a number of limitations. The number of placentas unavailable for examination represented one third of the entire study cohort. However, we are satisfied that the 668 twin pregnancies with pathological data available were representative of the overall study group. Complicated twin pregnancies do not appear to be overrepresented in this data set as the proportion of monochorionic and dichorionic twins was equal to that in the overall cohort. Of the total number of twin pregnancies recruited to the ESPRiT Study, 17.2% had >20% birthweight discordance. This is similar to the rate of 17.1% in this subgroup. The composite perinatal morbidity outcome occurred in 17.4% of this group, not significantly different to the rate of 18.2% in the overall cohort ($P = .6$). There were also no significant differences in gestational age at delivery, mean birthweight, or proportion of SGA twins in the 2 groups.

Another potential limitation is the fact that placental examination was carried out in the delivery hospital rather than in a single pathology laboratory. To minimize differences in placental reporting a

detailed protocol for placental examination was designed, the pathological lesions to be assessed were prespecified and a standardized data collection sheet was used. Other placental pathology findings including signs of inflammation and fetal vascular lesions were inconsistently reported between centers, thus the analysis was limited to the lesions prespecified in the study protocol.

In conclusion, in a large prospectively assessed cohort of monochorionic and dichorionic twin we have established an association between placental pathology and growth abnormalities. Together with our previously published data it adds further depth to our knowledge of the various pathological processes governing growth in twin pregnancies. ■

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